2697-Pos Board B389

Automated Detection and Analysis of Ca^{2+} Sparks in X-Y Image Stacks using a Novel Algorithm Implemented Within the Open-Source Image Analysis Platform, Imagej

Elliot M. Steele, **Derek S. Steele**.
School of Biomedical Sciences, Leeds, United Kingdom.

This study describes the development and characterisation of software to enable automatic detection and analysis of Ca^{2+} sparks within x-y image stacks, implemented as a plugin within the open source image analysis platform, ImageJ. The aim was to implement a "conventional" algorithm whereby sparks were identified by applying a threshold (θ) to the normalised (F/F_0) image: θ = background fluorescence within the cell + SD * 'ε', a user defined variable. A 2 stage interactive method with a graphical user interface (GUI) was used to ensure precise identification of the cell boundary and creation of a binary cell mask, which is subsequently used to exclude all regions outside the cell. The algorithm separates spark detection and analysis, allowing image processing to be applied independently at both stages. Filters also allow exclusion of events based on spark width or morphology. Novel methods are included to allow correction of time dependent changes in background fluorescence (e.g. due to bleaching), which would otherwise compromise spark detection by thresholding. The main outputs (amplitude, width, duration and spark mass) are presented in tabular form. In addition, an interactive GUI allows each spark to be examined, along with its measurements, and the associated Gaussian curve fit. A "Kill" button allows obvious errors in detection to be excluded. The performance of the algorithm was tested both on synthesised images (values of ε ranging from 3.0-4.2 and signal to noise ratios of 2, 3 or 4) and on x-y confocal fluorescence images from fluo-3 loaded rat ventricular myocytes. In both cases the performance was comparable to that reported previously for threshold based detection methods applied to line-scan images.

2698-Pos Board B390

Quantitative Analysis of Calcium Spikes in Noisy Fluorescent Background

Radoslav Janicek[1], **Matej Hotka**[1,2], Alexandra Zahradnikova, Jr[1], Ivan Zahradnik[1], Alexandra Zahradnikova[1].
[1]Department of Muscle Cell Research, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia, [2]Department of Biophysics, Faculty of Natural Sciences, Pavol Jozef Safarik University, Kosice, Slovakia.

Detection of local intracellular calcium signals in cardiac myocytes by laser-scanning confocal fluorescence microscopy requires high spatial and temporal resolution. It is not sufficiently understood how the resulting low signal-to-noise ratio (SNR) limits detection and description of calcium signals. We analyzed the accuracy and precision of description of calcium spikes by the theoretical equation [1] using synthetic datasets generated either with randomly varied spike parameters and Gaussian noise of constant amplitude, or with constant spike parameters and Gaussian noise of various amplitudes. The detection and analysis procedure was optimized for reliability of parameter estimation, maximization of the fraction of detected spikes, and minimization of the occurrence of false events. The acquisition time of individual pixels in line-scan images was corrected for errors introduced by sequential acquisition of pixels along the space coordinate. The relative error of parameter estimation and the fraction of undetected spikes increased above 10% for SNR < 2. In our experimental database, such a low SNR occurred only in 0.5% of spikes recorded with Fluo-3 and in 5% of spikes recorded with Oregon Green BAPTA-5N. The optimized software for analysis of calcium spikes written in MATLAB was provided for general use [2]. It allows interactive dissection of temporal profiles of calcium spikes from x-t images, their fitting with predefined function(s) and acceptance of results on statistical grounds, thus providing efficient analysis and reliable description of calcium signals in isolated cardiac myocytes down to the *in situ* activity of ryanodine receptors.

Supported by the grants VEGA 2/0197/11, VEGA 2/0203/11, and APVV-0721-10.

References:

[1] Zahradníková A Jr, Poláková E, Zahradník I, Zahradníková A. *J Physiol* 578: 677, 2007.
[2] Janicek R, Hotka M, Zahradníková A Jr, Zahradníková A, Zahradník I. *PLOS One* 8: e64394, 2013.

2699-Pos Board B391

Sparklab[2]: A Statistically Based Program to Detect 2D Sparks. Application to Smooth Muscle

Jose L. Puglisi, Manuel Navedo, Donald M. Bers, Leighton T. Izu.
Pharmacology, University of California, Davis, Davis, CA, USA.

We improved the statistical sieve algorithm developed by Izu et al. (Biophys. J. 92(12):4458-65) and simplified the user interface. In this new version, the normalized image (**Norm**) and background fluorescence is automatically calculated by the program. After reading the stack of images for a given cell, a template is created by averaging all the images. This template will constitute the norm against which each individual frame will be normalized. The cell border is detected from this template, and fluorescence outside this area is considered the background that is subtracted from all images. Then each frame is normalized by the **Norm** image. The next step highlights events as potential sparks, if fluorescence is ≥ 3 standard deviations from the **Norm** value. A final statistical test in the time domain is applied at each potential event. If the amplitude of the event is statistically different from the mean value of the previous 10 frames, the event is considered a spark. A table with the amplitude, FWHMx, FHHMy, Tau, Frame and location (x,y) is generated. Additionally the user can inspect each of the sparks in a 3D plot or its fitting profiles in x,y and t axis. A user friendly interface implemented in LabVIEW with intuitive menus guide the user through the whole process. We tested the functionality of the program by analyzing Ca^{2+} sparks from vascular smooth muscle. SparkLAB[2] presents the advantages of being a statistically based spark detector with minimum intervention of the user thus reducing the possibility of human bias.

2700-Pos Board B392

A Chloride Channel Blocker Prevents Inorganic Phosphate Accumulation and Its Effects in the Sarcoplasmic Reticulum of Frog Permeabilized Skeletal Muscle Fibers

Juan José Ferreira[1], Germán Pequera[1], Bradley Launikonis[2], Eduardo Ríos[3], Gustavo Brum[1].
[1]Facultad de Medicina, Montevideo, Uruguay, [2]School of Biomedical Sciences, University of Queensland., Brisbane, Australia, [3]Rush University, Chicago, IL, USA.

In skeletal muscle intense activity is accompanied by an increase in intracellular inorganic phosphate (Pi) due to breakdown of ATP and phoshocreatine. It is widely accepted that Pi enters the sarcoplasmic reticulum (SR) where it precipitates as Ca^{2+} salt, thus contributing to the impairment of Ca^{2+} release in muscle fatigue (Fryer et al, J Physiol 1995; Launikonis et al., Biophys J 2005). We studied the effects of increasing Pi concentrations on elementary Ca^{2+} release events (sparks) in permeabilized fibers. Spark frequency F/F0 increased with [Pi] up to 7-10 mM/l and decreased monotonically reaching 0.1 at [Pi] = 55 mM. These changes were highly correlated with the level of $[Ca^{2+}]$SR, monitored with Fluo5N or Mag-Fluo4. SR anion channels, probably chloride channels (Laver et al.,J Physiol2001), provide the principal entry path of Pi to the SR. The chloride channel blocker 9-anthracenecarboxylic acid (9AC, at 400 uM) increased spark frequency by about 15% and shifted the Pi effects to slightly higher concentrations. Because 9AC blocks the channel from the SR-luminal side another approach was taken to enhance its action. Fibers were incubated in the presence of 5 mM Mg^{2+} and 2 mM [9AC] for 30 minutes. There after 9AC was reduced to 400 uM and Mg^{2+} to 0.4 mM/l. After this protocol, Pi concentrations up to 55 mM/l had no effect on frequency or spark morphology. Correspondingly, $[Ca^{2+}]$SR-monitoring signals were not affected. These results indicate that chloride channels constitute the main entrance of Pi to the SR, confirm that Pi impairs Ca^{2+} release by accumulating and precipitating with Ca^{2+} inside the SR and provide approaches to study these mechanisms quantitatively.

Funded by CSIC and NIH.

2701-Pos Board B393

The Mboat Family Protein Mitsugumin 56 Contributes to Postnatal Maturation in the Muscle Sarcoplasmic Reticulum

Bo Van[1], Miyuki Nishi[1], Shinji Komazaki[2], Ki Ho Park[3], Daiju Yamazaki[1], Jianjie Ma[3], Hiroshi Takeshima[1].
[1]Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan, [2]School of Medicine, Saitama Medical University, Saitama, Japan, [3]Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH, USA.

The sarcoplasmic reticulum (SR) is a highly-specialized form of the smooth endoplasmic reticulum (ER), and functions intensively as an intracellular Ca^{2+} store in muscle cells. Although major proteins contributing to Ca^{2+} handling have been extensively studied, there are still as-yet-unknown SR components, which would potentially open new fields in muscle physiology. We currently identified mitsugumin 56 (MG56), that is a novel SR membrane protein expressed in striated muscle. MG56 belongs to the MBOAT (membrane-bound O-acyltransferase) family, and is specifically localized to the junctional SR composing the triad in skeletal muscle. Although *Mg56*-knockout mice normally grew until postnatal day 7 (P7), they exhibited suckling failure and died within two weeks under starvation conditions. In skeletal muscle from *Mg56*-knockout mice, SR elements began to swell near the Z-line on P5, and further developed enormous vacuoles spreading over sarcomeres afterwards. In tension measurements, *Mg56*-knockout extensor digitorum